Instruction Manual

# Thermo Scientific KingFisher Pure RNA Blood Kit

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**NOTE:** For more details on storing the kit reagents, refer to "Storage Conditions" on page 6.

# 1

# Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure RNA Blood Kit

| Item | KingFisher Pure RNA Blood Kit |
|---|---|
| Cat. No. | 98020196 |
| Package size | 96 samples |
| Lysis Buffer | 27 ml |
| Proteinase K | 2 x 1.0 ml |
| KingFisher Magnetic Beads | 1 x 1.6 ml |
| Rebinding Buffer (conc.)* | 25 ml |
| Wash Buffer 1 (conc.)* | 100 ml |
| Wash Buffer 2 (conc.)* | 30 ml |
| DNase I (lyophilized) | 2 vials |
| DNase I Reconstitution Buffer | 2 x 1.0 ml |
| Manganese Chloride Solution | 1 ml |
| 2 x DNase I Buffer | 3 x 1.5 ml |
| Nuclease-free water | 30 ml |

* Addition of ethanol or isopropanol required.

The KingFisher Pure RNA Blood Kit (Cat. No. 98020196) is intended for the purification of blood samples, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head and a sample volume of 200 µl.

The user will need the KingFisher Flex or KingFisher Duo magnetic particle processor for conducting purification (Table 1-2). In addition, several common laboratory instruments and consumables are necessary to conduct

an efficient purification. For more details, refer to Chapter 5: "Protocols and Pipetting Instructions". Suitable consumables for the KingFisher Duo and KingFisher Flex are listed in Table 1-3 and Table 1-4.

## Storage Conditions

Upon arrival of the kit, store the DNase I, DNase I Reconstitution Buffer, and 2 x DNase Buffer at -20°C. Also after dissolving of the DNase I, continue to keep it at -20°C. The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. Store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to two years from the manufacturing date.

## Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade
- 100% isopropanol, molecular biology grade
- 2 M DTT (dithiothreitol) solution (store at -20°C)
- PBS (Phosphate buffered saline for Tempus™ protocol)

Table 1-2. Thermo Scientific™ KingFisher™ magnetic particle processors

| Cat. No. | Product |
|---|---|
| 5400100 | KingFisher Duo magnetic particle processor |
| 5400630 | KingFisher Flex magnetic particle processor with 96 deep well head |

Table 1-3. Thermo Scientific™ KingFisher™ Flex consumables

| Cat. No. | Product | Package size |
|---|---|---|
| 97002534 | KingFisher Flex 96 tip comb for deep well magnet | 100 pcs |
| 97002540 | KingFisher Flex 96 KF plate (200 µl) | 48 pcs |
| 95040450 | Microtiter deep well 96 plate | 50 pcs |
| 95040460 | Microtiter deep well 96 plate, sterile | 50 pcs |

Table 1-4. Thermo Scientific™ KingFisher™ Duo consumables

| Cat. No. | Product | Package size |
|---|---|---|
| 97003500 | KingFisher Duo 12-tip comb for Microtiter deep well 96 plate | 50 pcs |
| 97003520 | KingFisher Duo elution strip | 40 pcs |
| 95040450 | Microtiter deep well 96 plate | 50 pcs |
| 95040460 | Microtiter deep well 96 plate, sterile | 50 pcs |
| 97003530 | KingFisher Duo Combi pack for Microtiter deep well 96 plate<br>(tip combs, plates, and elution strips for 96 samples) | 1 box |

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# Product Description

## Introduction

The KingFisher Pure RNA Blood Kit is designed for rapid automated RNA purification from up to 200 µl of mammalian blood samples using Thermo Scientific™ KingFisher™ instruments. See the detailed list below.

1) Fresh or frozen whole human blood samples anticoagulated by EDTA (ethylenediaminetetraacetic acid) or citrate, and samples stabilized by commercial stabilizers PAXgene or Tempus

2) Fresh, frozen, or stabilized (PAXgene and Tempus) blood of different species: mouse, rabbit, canine, horse, cow, sheep, and goat

3) Human buffy coat, blood leucocytes, erythrocytes, and bone marrow samples

The RNA purified using the KingFisher Pure RNA Blood Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as RT-qPCR (reverse transcription quantitative PCR), RT-PCR, and several other enzymatic reactions.

## Intended Use

The KingFisher Pure RNA Blood Kit is developed for the purification of RNA from blood samples using paramagnetic particles. The reagents and specific plastic consumables are designed for use with the KingFisher Flex and KingFisher Duo magnetic particle processors as part of an integrated system. The KingFisher Pure RNA Blood Kit enables extraction of RNA from fresh or stored blood. The KingFisher Pure RNA Blood Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the

Thermo Scientific™ KingFisher™ instrument and the KingFisher Pure RNA Blood Kit for any particular use, as the performance of the kits has not been validated for any specific organism or downstream application.

## Principle and Procedure

The KingFisher Pure RNA Blood Kit uses magnetic-particle technology for RNA purification. The Thermo Scientific™ KingFisher™ technology combines the speed and efficiency of RNA purification with easy handling of magnetic particles. The purification process requires no phenol/chloroform extraction and needs very little hands-on time.

The first step of the protocol lyses the cells, after which the NA (nucleic acid) can bind to the surface of the KingFisher Magnetic Beads in the presence of a chaotropic salt. Copurified DNA is removed during a DNase I treatment step.

The following effective wash steps dispose of proteins, cell debris, and any residual contaminants, while the RNA bound to the KingFisher Magnetic Beads is transferred through the wash steps.

Two different Wash Buffers are used, followed by an air drying step. High-quality RNA is eluted into nuclease-free water, and is ready for subsequent downstream processes.

## Kit Specifications

The KingFisher Pure RNA Blood Kit is designed for rapid automated preparation of highly pure total RNA from up to 200 µl of blood samples using KingFisher magnetic particle processors.

If a dispense step requiring the addition of Rebinding Buffer is excluded, the approximate processing time is 45 min for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo. The obtained RNA can be used directly in various downstream applications.

Fresh or stored blood samples can be used. Suitable sample storage is essential for obtaining a high yield and good quality total RNA.

Typically 1.5-4 µg of total RNA can be purified from a 200 µl volume of blood sample with an $A_{260}/A_{280}$ ratio of ≥ 1.8–2.1. The yields of acquired purified RNA depend on the sample type, and the method of sample collection, and storage. Refer to Appendix B for typical total RNA yields from various sources.

## KingFisher Magnetic Particle Processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific™ KingFisher™ systems is based on the use of magnetic rods covered with a disposable, specially designed tip comb and plates or tubes. Use only Thermo Scientific™ KingFisher™ plastic consumables, as use of products from other manufacturers may cause unsuitable mixing or even instability in the KingFisher instrument. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents, including magnetic particles, are dispensed onto the plates according to the corresponding instructions. Dispensing can be carried out manually or partially automatically using automatic dispensers, for example, the Thermo Scientific™ Multidrop™ Combi and/or the Thermo Scientific™ Versette™. Thermo Scientific™ BindIt™ Software 3.2 can be used for running ready-made and optimized protocols for the Thermo Scientific™ KingFisher™ Pure Kits. It is also possible to transfer the developed protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments provide a rapid and automated solution for complicated and time-consuming purification processes, resulting in high-purity RNA without risk of carryover or cross-contamination.

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables, and the easy-to-use BindIt Software 3.2. The KingFisher Pure RNA Blood Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

KingFisher magnetic particle processors are intended for professional research use by trained personnel. Detailed information and user instructions for the KingFisher instruments can be found in their respective user manuals.

The BindIt Software 3.2 protocols optimized for the KingFisher Pure RNA Blood Kit are available for the KingFisher Flex 96 and 24 and KingFisher Duo. For more information, go to www.thermoscientific.com/kingfisherinfo or contact your local authorized distributor.

Table 2-1. Overview of KingFisher Flex and KingFisher Duo magnetic particle processors

| | KingFisher Flex | | KingFisher Duo | |
|---|---|---|---|---|
| | 96 deep well format | 24 format | 12 format | 6 format |
| Processing volume | 50–1000 µl* | 200–5000 µl | 50–1000 µl* | 200–5000 µl |
| Capacity | Up to 96 samples per run (sample volume approx. 200 µl) | Up to 24 samples per run (sample volume approx. 1 ml) | Up to 12 samples per run (sample volume approx. 200 µl) | Up to 6 samples per run (sample volume approx. 1 ml) |
| Magnetic head | 96 inter-changeable formats for Microtiter deep well 96 plate, PCR plate and KingFisher Flex 96 KF plate | 24 format for KingFisher Flex 24 deep well plate | 12-pin magnet head for Microtiter deep well 96 plate | 6-pin magnet head for KingFisher Flex 24 deep well plate |
| Plates | KingFisher Flex 96 KF plate (20–200 µl), 96 well PCR plate, skirted (20–100 µl), Microtiter deep well 96 plate (50–1000 µl) | KingFisher Flex 24 deep well plate (200–5000 µl) | Microtiter deep well 96 plate (50–1000 µl), KingFisher Duo elution strip (30–130 µl) | KingFisher Flex 24 deep well plate (200–5000 µl) |
| Tip combs | KingFisher Flex 96 tip comb for PCR magnets, KingFisher Flex tip comb for KF magnets, KingFisher Flex 96 tip comb for deep well magnets | KingFisher Flex 24 tip comb for deep well magnets | KingFisher Duo 12-tip comb | KingFisher Duo 6-tip comb |
| Heating temperature | Heating block temperature from +5°C above ambient room temperature to +115°C | | Heating block temperature from +10°C to +75°C, elution strip +4°C to +75°C at room temperature | |

* See the details above on the Plates row.

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# Safety Information

The following components of the KingFisher Pure RNA Blood Kit contain hazardous contents (Table 3-1).

Always wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions provided in the kit instruction manual. It is recommended that Good Laboratory Practice (GLP) is followed to guarantee reliable analyses.

Table 3-1. Safety precautions

| Reagent | Hazardous contents | Safety instructions |
|---|---|---|
| Lysis Buffer | Guanidium thiocyanate | Harmful by inhalation. Liberates very toxic gas in contact with acids. Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment.<br>Keep the container in a well-ventilated place. Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/safety data sheets. |

Continued

Cont.

| Reagent | Hazardous contents | Safety instructions |
|---|---|---|
| Proteinase K | Proteinase, Tritirachium album serine | May cause sensitization by inhalation.<br>Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately. This material and its container must be disposed of as hazardous waste. |
| Rebinding Buffer (conc.) | Sodium perchlorate | Harmful if swallowed. Explosive when mixed with combustible material. Keep away from combustible material. Do not breathe gas/fumes/vapor/spray. Take off immediately all contaminated clothing. Wear suitable protective clothing. This material and its container must be disposed of as hazardous waste. |
| Wash Buffer 1 (conc.) | Guanidium chloride | Harmful if swallowed. Irritating to eyes and skin.<br>Do not breathe gas/fumes/vapor/spray. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste. |

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# Storage Conditions and Preparation of the Reagents

## Storage Conditions

Upon arrival of the kit, store the DNase I, DNase I Reconstitution Buffer, and 2 x DNase Buffer at -20°C. The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. Store the KingFisher Magnetic Beads at +4°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to two years from the manufacturing date.

## Preparation of the Lysis Buffer

Before each RNA purification experiment, a fresh aliquot of Lysis Buffer should be supplemented with 2 M DTT solution (not provided). It is required to use 200 µl of Lysis Buffer for the purification of one sample. Add 20 µl of 2 M DTT to 1 ml of Lysis Buffer.

## Preparation of the Wash Buffers and Rebinding Buffer

Add 100% isopropanol and 96–100% ethanol to the Wash Buffer 1, Wash Buffer 2, and Rebinding Buffer, as indicated below in Table 4-1 prior to the first use.

Table 4-1. Instructions for the preparation of Wash Buffer 1, Wash Buffer 2, and Rebinding Buffer. Add the indicated volume of 100% isopropanol or 96–100% ethanol to each bottle.

| | Wash Buffer 1 | Wash Buffer 2 | Rebinding Buffer |
|---|---|---|---|
| Concentrated buffer | 100 ml | 30 ml | 25 ml |
| Ethanol (96–100%) | – | 120 ml | – |
| Isopropanol (100%) | 90 ml | – | 33.4 ml |
| Total volume | 190 ml | 150 ml | 58.4 ml |

After preparing each solution, mark the bottle to indicate that the step has been completed. The buffers can be stored at room temperature.

## Preparation of the DNase I Storage Solution

To prepare the DNase I storage solution, add 1100 µl of DNase I Reconstitution Buffer to each vial of lyophilized DNase I. Incubate at room temperature for 5 min. Occasional gentle rotation of the vial helps to dissolve the DNase I, but avoid forceful mixing. Store the DNase I storage solution at -20°C. Repeated freezing and thawing should be avoided.

## Preparation of the DNase I Working Solution

Calculate the amount of DNase I working solution needed. For the purification of one sample, mix 35 µl of 2 x DNase I Buffer, 1.5 µl Manganese Chloride Solution, 10 µl of DNase I storage solution, and 23.5 µl of nuclease-free water. The DNase I working solution should be used immediately after preparation.

## Starting Material Handling and Storage

RNA purification from blood is recommended to be carried out on the day of collection. Fresh blood samples collected in anticoagulants (ethylenediaminetetraacetic acid (EDTA) or citrate) containing vials are not recommended to be stored at +4°C for longer than six hours. Aliquots of fresh blood samples, if frozen immediately, can be stored at -80°C for several months.

Blood samples collected in PAXgene or Tempus tubes should be stored according to the manufacturer's instructions.

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# Protocols and Pipetting Instructions

Before beginning the RNA purification protocol, carefully read through the *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669), the *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. No. N12420) or the *Thermo Scientific™ KingFisher™ mL User Manual* (Cat. No. 1508260), and the *Thermo Scientific™ BindIt™ Software for KingFisher Instruments version 3.2 User Manual* (Cat. No. N07974).

BindIt Software protocols for the KingFisher Pure RNA Blood Kit can be found in BindIt Software 3.2 and at www.thermoscientific.com/kingfisher.

## Handling of KingFisher Magnetic Beads

A homogeneous distribution of the KingFisher Magnetic Beads in the container is essential before the beads are transferred to the wells or tubes in order to ensure a high consistency between the wells or tubes. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly.

## Avoiding Ribonuclease (RNase) Contamination

RNA purity and integrity is essential for downstream applications. RNase is a ubiquitously found enzyme, which degrades RNA. RNases are highly stable contaminants found in any laboratory environment. Keep all kit components tightly sealed when not in use.

Skin is a common source of RNases. Always wear gloves when handling reagents and RNA samples. Use sterile, RNase-free pipette tips when working with RNA. Remove RNase contamination from work surfaces and non-disposable items (pipettes, centrifuges) with reagents designed to remove RNase.

## Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for RNA purification from 200 µl of whole blood, using the KingFisher Pure RNA Blood Kit (Cat. No. 98020196) and the KingFisher Flex with Thermo Scientific™ Microtiter™ deep well 96 plates.

When using the KingFisher Pure RNA Blood Kit for the first time, prepare the DNase I storage solution, Rebinding Buffer, Wash Buffer 1, and Wash Buffer 2. For each run, prepare the DNase I working solution and Lysis Buffer with 2 M DTT. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

Blood samples of certain species, e.g. mouse, contain a high amount of RNA. With such species, it is recommended to use up to 50 µl of blood sample to obtain RNA of equal yield and purity. When working with less than a 200 µl volume of blood sample, it is recommended to equalize the volume by adding Lysis Buffer, thus bringing the total volume to 400 µl.

1. Take six empty Microtiter deep well 96 plates and two empty Thermo Scientific™ KingFisher™ Flex 96 KF plates.

2. Fill the **plates** as follows.

| Plate number | Plate type | Plate name | Content | Reagent volume per well |
|---|---|---|---|---|
| 1 | Microtiter deep well 96 plate | Sample | Lysis Buffer<br>Blood sample<br>Proteinase K | 200 µl<br>200 µl<br>20 µl |
| 2 | | Wash 1_1 | Wash Buffer 1 | 900 µl |
| 3 | | DNase | DNase I working solution | 70 µl |
| 4 | | Wash 1_2 | Wash Buffer 1 | 700 µl |
| 5 | | Wash 2_1 | Wash Buffer 2 | 700 µl |
| 6 | | Wash 2_2 | Wash Buffer 2 | 700 µl |
| 7 | KingFisher Flex 96 KF plate | Elution | Nuclease-free water | 75 µl |

3. Place a Thermo Scientific™ KingFisher™ Flex 96 tip comb for deep well magnets on a **Tip Plate** (i.e. an empty KingFisher Flex 96 KF plate).

4. Start the PURE_RNABlood_Flex96 protocol using the KingFisher Flex 96 and load the plates as instructed on the KingFisher Flex 96 instrument display.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 96 deep well head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the PURE_RNABlood_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

5. When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE_RNABlood_Flex96 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and isopropanol to the Sample plate during the first dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the Sample plate from the instrument, and add the KingFisher Magnetic Beads and isopropanol to the **Sample plate**.

| Plate name | Add | Added reagent volume per well |
|---|---|---|
| Sample | Isopropanol | 400 µl |
| | KingFisher Magnetic Beads* | 15 µl |

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Sample plate back into the instrument and press **Start**.

8. Add the Rebinding Buffer (supplemented with isopropanol) to the Sample plate during the second dispense step.

When the KingFisher Flex pauses at the second dispense step after the DNase step at approximately 10 minutes after the first dispense step, remove the DNase plate from the instrument, and add the Rebinding Buffer (supplemented with ethanol) to the **DNase plate** to rebind the RNA.

| Plate name | Add | Added reagent volume per well |
|---|---|---|
| DNase | Rebinding Buffer | 490 µl |

9. Place the DNase plate back into the instrument and press **Start**. After the pause, the protocol will continue to completion.

10. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. The purified RNA is ready for use in downstream applications. When working with RNA, keep the purified samples on ice. Store the purified RNA at -20°C or -80°C.

**NOTE:** The final RNA concentration in the nuclease-free water may increase if the purified RNA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

## Instructions for KingFisher Duo with 12-pin Magnet Head

These instructions are intended for RNA purification from 200 µl of blood, using the KingFisher Pure RNA Blood Kit (Cat. No. 98020196) and the KingFisher Duo with 12-pin magnet head.

When using the KingFisher Pure RNA Blood Kit for the first time, prepare the DNase I storage solution, Rebinding Buffer, Wash Buffer 1, and Wash Buffer 2. For each run, prepare the DNase I working solution and Lysis Buffer with 2 M DTT. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

1. Take one empty Microtiter deep well 96 plate and one Thermo Scientific™ KingFisher™ Duo elution strip.

2. Prepare the **Blood RNA plate** (i.e. a Microtiter deep well 96 plate). Add the following reagents to the rows. Note that row B is reserved for the tip comb and should be left *empty*. Note that row D is also left *empty*.

| Plate name and type | Row | Row name | Content | Sample/reagent volume per well |
|---|---|---|---|---|
| Blood RNA plate<br>Microtiter deep well 96 plate | A | DNase | DNase I working solution | 70 µl |
| | B | Tip | 12-tip comb | Empty |
| | C | Empty | Empty | Empty |
| | D | Wash 2_2 | Wash Buffer 2 | 700 µl |
| | E | Wash 2_1 | Wash Buffer 2 | 700 µl |
| | F | Wash 1_2 | Wash Buffer 1 | 700 µl |
| | G | Wash 1_1 | Wash Buffer 1 | 900 µl |
| | H | Sample | Lysis Buffer<br>Blood sample<br>Proteinase K | 200 µl<br>200 µl<br>20 µl |

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the nuclease-free water is pipetted into the correct wells.

| Elution strip | Content | Reagent volume per well |
|---|---|---|
| KingFisher Duo elution strip | Nuclease-free water | 75 µl |

4. Place a Thermo Scientific™ KingFisher™ Duo 12-tip comb into **row B** on the **Blood RNA plate**.

5. Start the PURE_RNABlood_Duo protocol using the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 12-pin magnet head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the PURE_RNABlood_Duo protocol. Insert the Blood RNA plate and elution strip into the instrument as indicated on the KingFisher Duo display and press **OK**.

Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

When the KingFisher Duo is to be run as a standalone instrument, transfer the PURE_RNABlood_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and isopropanol to row C during the dispense step.

When the KingFisher Duo pauses at the first dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the plate from the instrument, and add the KingFisher Magnetic Beads and isopropanol to **row C** on the **Blood RNA plate**.

| Row | Row name | Add | Added reagent volume per well |
|---|---|---|---|
| C | Sample | Isopropanol<br>KingFisher Magnetic Beads* | 400 µl<br>15 µl |

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the plate back into the instrument and press **OK**.

8. Add the Rebinding Buffer (supplemented with ethanol) to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step at approximately 10 minutes after the first dispense step, remove the plate from the instrument, and add the Rebinding Buffer into **row A** on the **Blood RNA plate** to rebind the RNA.

| Row | Row name | Add | Added reagent volume per well |
|---|---|---|---|
| A | DNase | Rebinding Buffer | 490 µl |

9. Place the plate back into the instrument and press **OK**. After the pause, the protocol will continue to completion.

10. After the run is completed, remove the plate and elution strip, and store the purified RNA.

When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and switch off the instrument. The purified RNA is ready for use in downstream applications. When working with RNA, keep the purified samples on ice. Store the purified RNA at -20°C or -80°C.

**NOTE:** The final RNA concentration in the nuclease-free water may increase if the purified RNA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

## Instructions for Total RNA Purification from PAXgene Stabilized Blood Samples

1. Collect and store the blood samples in PAXgene tubes according to the manufacturer's instructions.

2. If the PAXgene samples were stored in the cold, equilibrate the samples at room temperature before purification according to the manufacturer's instructions for approx. 2 h.

3. Centrifuge the PAXgene blood RNA tubes for 10 min at 3,000–5,000 x g.

4. Carefully discard the supernatant from each tube and wash the pellet. Add 1–3 ml of nuclease-free water, vortex for 20–30 s, and collect the pellet again by centrifuging for 10 min at 3,000–5,000 x g.

   - For 0.5–2 ml of PAXgene blood lysate, wash the obtained pellet with 1 ml of water.
   - For a whole tube of PAXgene blood lysate, wash the obtained pellet with 3 ml of water.

5. Carefully discard the supernatant, add 100 µl of nuclease-free water, and vortex for 10–20 s to suspend precipitates. Add 400 µl of Lysis Buffer (supplemented with DTT) and 20 µl of Proteinase K. Mix the lysate and continue the purification as in "Instructions for KingFisher Flex with 96 Deep Well Plates" on page 18 or as in "Instructions for KingFisher Duo with 12-pin Magnet Head" on page 20.

## Instructions for Total RNA Purification from Tempus Stabilized Blood Samples

1. Collect and store the blood samples in Tempus tubes according to the manufacturer's instructions.

2. If the sample is frozen, thaw the sample in the Tempus tube at room temperature according to the manufacturer's instructions.

3. Transfer the entire contents of the Tempus tube into a 50 ml conical tube. Bring the total volume to 12 ml by adding the required amount of PBS. Typically a Tempus stabilized blood sample volume is 9 ml, which requires the addition of 3 ml of PBS to bring the total volume to 12 ml.

4. Vortex efficiently for 30 s to ensure proper mixing of the contents.

5. Centrifuge the appropriate volume (1–2 ml) of Tempus blood lysate in conical tubes at maximal speed (about 12,000–16,000 × g) for 10 min at 4°C. If the Tempus blood volume is more than 2 ml, increase the time of centrifugation up to 30 min.

6. Carefully discard the supernatant from each tube and add 450 µl of Lysis Buffer (supplemented with DTT) and 20 µl of Proteinase K. Vortex the samples and incubate at room temperature for 10 min. Continue the purification as in "Instructions for KingFisher Flex with 96 Deep Well Plates" on page 18 or as in "Instructions for KingFisher Duo with 12-pin Magnet Head" on page 20.

## Quantification and Determination of the Purity of RNA

It is recommended to measure the absorbance at 320 nm, 280 nm, and 260 nm. The concentration of RNA can be defined with the absorbance at 260 nm ($A_{260}$). One unit at 260 nm corresponds to 40 µg of RNA per ml. The ratio between the $A_{260}/A_{280}$ indicates the purity of the RNA. The value for RNA should be ≥ 1.8–2.1.

It is recommended that $A_{320}$ correction is used for the absorbance values. Subtract the $A_{320}$ from the $A_{260}$ and $A_{280}$ ratios to remove the effects of carryover of the magnetic particles.

- Concentration of RNA sample = 40 µg/ml x ($A_{260} - A_{320}$) x dilution factor
- Total amount of RNA isolated = concentration x volume of sample in ml
- Purity of RNA sample = ($A_{260} - A_{320}$)/( $A_{280} - A_{320}$)

6

# General Information

## Reagent Specificity and Volumes

A reagent must not be used with any kit other than that for which it is intended. It is strongly recommended that the volume of reagents in each well or tube is kept within the limits specified in the *KingFisher Flex User Manual* or *KingFisher Duo User Manual* to avoid spillover and to maximize efficiency of performance.

## Handling of Magnetic Beads

The KingFisher Magnetic Beads should be mixed thoroughly before use to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes. The amount of beads in the wells or tubes affects the yield of the purified RNA.

## Binding, Wash, and Elution Steps

The binding between the purified RNA and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt.

The volume of the Elution Buffer can be modified depending on user requirements concerning the purified RNA concentration. The final RNA concentration in the Elution Buffer may increase if the purified RNA is eluted into a smaller than recommended volume of the buffer, but this can slightly reduce the overall RNA yield. The modifications of the elution step must be done in BindIt Software 3.2 and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

Table 6-1. Available elution volumes of the KingFisher Flex and KingFisher Duo

| KingFisher instrument | Elution volumes |
|---|---|
| KingFisher Flex with 96 deep well head, elution in a KingFisher Flex 96 KF plate | 50–150 µl |
| KingFisher Flex with 96 deep well head, elution in a Microtiter deep well 96 plate | 50–1000 µl |
| KingFisher Flex with 24 deep well head | 200–5000 µl |
| KingFisher Duo with 12-pin magnet head, elution in an elution strip | 30–130 µl |
| KingFisher Duo with 12-pin magnet head, elution in a Microtiter deep well 96 plate | 50–1000 µl |

To maximize the yield of purified RNA, avoid the lowest permitted volumes of Elution Buffer in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely, and any possible sedimented magnetic-bead pellet(s) should be completely resuspended. In addition, the volume of the Elution Buffer should be adequate for efficient mixing of the beads in order to obtain a maximal release of the purified RNA from the beads.

## Decontamination and Disinfection of Sample Material

You should decontaminate the sample material and the reagents and plastics that have been in contact with the sample material in order to minimize the risk of contamination. Use a decontaminant, such as Virkon™, paying due attention to the manufacturer's instructions. You should also take care of the appropriate treatment and/or disposal of waste.

A

# Troubleshooting

| Problem | Possible cause and actions |
|---|---|
| Low RNA yield | There should be an adequate volume of the Elution Buffer to cover the KingFisher Magnetic Beads completely during the elution step.<br>Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency.<br>Prolonged storage of the sample material may reduce the total RNA yield.<br>Use only Thermo Scientific plates, strips, and tip combs with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified RNA. |
| Low purity | Prolonged storage of the sample material may reduce the quality and quantity of the total RNA.<br>Insufficient washing causes impurities in the eluted RNA. |

Continued

Cont.

| Problem | Possible cause and actions |
|---|---|
| Magnetic particles remaining in the sample or elution well | Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the particles unless the viscose samples are diluted before the beginning of the purification process. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads.<br><br>If the KingFisher Magnetic Beads are inefficiently collected from the Elution Buffer, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results.<br><br>KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the total RNA yield, as the RNA has already been released from the KingFisher Magnetic Beads into the Elution Buffer.<br><br>If the KingFisher magnetic particle processor does not work properly, refer to the relevant user manual of the KingFisher instrument in use. |

B

# Typical RNA Yields

Table B-1. Typical total RNA yields from whole mammalian blood of different species using KingFisher magnetic particle processors

| | Source | Yield of RNA (µg/ml blood) |
|---|---|---|
| 1. | Fresh human blood | 11–20 |
| 2. | Frozen human blood | 7–17 |
| 3. | Stabilized (PAXgene, Tempus) human blood | 9–12 |
| 4. | Human bone marrow | 15–80 |
| 5. | Fresh mouse blood | 120–160 |
| 6. | Fresh rabbit blood | 25–30 |
| 7. | Fresh canine blood | 11–15 |
| 8. | Fresh animal (horse, cow, sheep, goat) blood | 2–5 |

C

# Ordering Information

Table C-1. KingFisher Pure RNA Blood Kits

| Cat. No. | Product | Package size |
|---|---|---|
| 98020196 | KingFisher Pure RNA Blood Kit | 96 |

Table C-2. KingFisher Flex consumables

| Cat. No. | Product | Package size |
|---|---|---|
| 97002514 | KingFisher Flex 96 tip comb for PCR magnet | 80 pcs |
| 97002524 | KingFisher Flex 96 tip comb for KF magnet | 100 pcs |
| 97002534 | KingFisher Flex 96 tip comb for deep well magnet | 100 pcs |
| 97002610 | KingFisher Flex 24 deep well tip comb and plate | 50 pcs |
| 97002540 | KingFisher Flex 96 KF plate (200 µl) | 48 pcs |
| 95040450 | Microtiter deep well 96 plate | 50 pcs |
| 95040460 | Microtiter deep well 96 plate, sterile | 50 pcs |
| 95040470 | KingFisher Flex 24 deep well plate | 50 pcs |
| 95040480 | KingFisher Flex 24 deep well plate, sterile | 50 pcs |

Table C-3. KingFisher Duo consumables

| Cat. No. | Product | Package size |
|---|---|---|
| 97003500 | KingFisher Duo 12-tip comb for Microtiter deep well 96 plate | 50 pcs |
| 97003510 | KingFisher Duo 6-tip comb for KingFisher Flex 24 deep well plate | 48 pcs |
| 97003520 | KingFisher Duo elution strip | 40 pcs |
| 95040450 | Microtiter deep well 96 plate | 50 pcs |
| 95040460 | Microtiter deep well 96 plate, sterile | 50 pcs |
| 95040470 | KingFisher Flex 24 deep well plate | 50 pcs |
| 95040480 | KingFisher Flex 24 deep well plate, sterile | 50 pcs |
| 97003530 | KingFisher Duo Combi pack for Microtiter deep well 96 plate<br>(tips combs, plates, and elution strips for 96 samples) | 1 box |

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